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VECTOR COMPETENCE OF MOSQUITOES FOR ARBOVIRUSES

Annual Report

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The extreme peroral refractoriness of Aedes aegypti to dengue 2 (New Guinea C) viral infection can be mollified by using freshly grown virus. Infection rates were increased if mosquitoes were allowed to feed to engorgement on a mixture of concentrated infected cells and infectious supernatant. The infectious supernatants were concentrated approximately 10-fold in Centricon® filters and this proved to further enhance peroral infection rates. The dose required to infect 50% of the Rockefeller strain of Ae. aegypti, following feeding on a pledget, has now been more accurately estimated to be 8×10^8 PFU/ml ingested.

Two monoclonal antibodies to mosquito, Culex tarsalis, mesenteronal brush border proteins have been further characterized. Antibody 13A5.8 has been used in minicolumns to isolate specific antigens from both western equine encephalomyelitis virus susceptible (WS) and refractory (WR) mesenteronal brush border fragments (BBF). These related antigens are termed R2 (refractory) and S2 (susceptible). Another antibody (32G8) has revealed a relationship between R2 and S2 that suggests posttranslational modification of a nascent (R2)(over)

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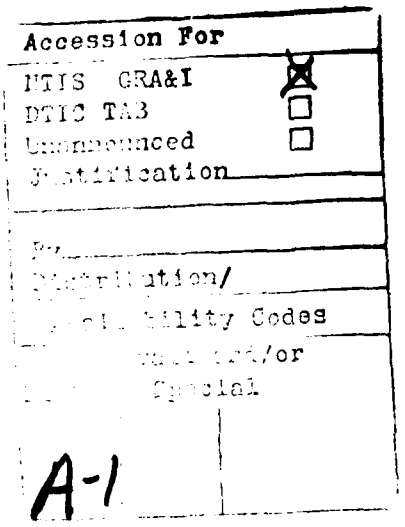
protein. 32G8 reacts against the R2 antigen and a small protein fragment,50-70 kDal, in susceptible mesenterons.

Cell culture studies with three strains of Aedes albopictus cells (U4.4, Singh and C6/36) have revealed that U4.4 and Singh cells are both capable of modulating alphaviral titers. This modulation is eliminated following treatment with alpha-amanitin.

The congenic (L.H) line of Cx. tarsalis, which was selected as a nonmodulating line from the LVP strain (Modulating), did not yield the anticipated patterns of peroral and parenteral infection. The L.H line appears to have been selected for receptor sites in mesenteronal, and perhaps other tissues, membranes.



I. Summary

The peroral infection of *Aedes aegypti* females with dengue 2 (DEN2) virus presented in an artificial bloodmeal is proven to be extremely difficult. Two approaches to circumvent this problem were undertaken: 1) alteration of bloodmeal composition and 2) concentration of DEN2 virus to increase the titer of infectious bloodmeals.

The composition of artificial infectious bloodmeals has been quite varied, as reported in the literature. Recent studies have shown that one of the most infectious composition was the cell pellet from the centrifugation of infected C6/36 cells resuspended in infectious cell culture supernatant. The resuspended pellet (0.1 ml) was mixed with freshly washed red blood cells (0.4 ml), fetal calf serum (0.45 ml) and 60% (w/v) sucrose (0.05 ml). Other bloodmeals have been prepared from frozen infected cell culture supernatants, fresh infected cell culture supernatants and parenterally infected mosquito homogenates. All of these means of preparing an infectious artificial bloodmeal were examined. In addition, the infectiousness of BHK-21 and C6/36 cell culture derived DEN2 virus was compared.

The results corroborated previous reports from the literature. Infectious artificial bloodmeals prepared from the pellets of infected cell cultures were the most infectious. There was litttle difference between the mosquito infection rates when they were fed on bloodmeals prepared from C6/36 cells (82) or BHK-21 cells (73%).

An alternative or supplemental approach to increasing the infectiousness of artificial bloodmeals might be through an increase in viral titers by concentrating the supernatants from infected cell cultures. Centricon® filters are available which allow one to rapidly, but gently, concentrate virions, or proteins >30,000 kD, up to 40-fold. Using these filters, the volume of infected C6/36 cell culture supernatants was routinely increased by approximately 10-fold. The increase in DEN2 viral titer was essentially equivalent to the level of volume concentration.

The infectiousness of concentrated supernatant and infected C6/36 cell culture pellets resuspended in either concentrated or unconcentrated supernatants was compared. Concentrated supernatant was no more infectious than unconcentrated supernatant had been in previous experiments. Infected cell culture pellets resuspended in concentrated cell culture supernatants appeared to be slightly more infectious than cell culture pellets resuspended in

unconcentrated cell culture supernatants. However, the difference was not statistically significant.

The peroral infectious dose to infect 50% (ID_{50}) of the Rexville and Rockefeller strains of *Ae. aegypti* was established. The Rockefeller strain was slightly more susceptiblewith an ID_{50} of $8x10^8$ PFU/ml ingested, compared to $1.7x10^9$ PFU/ml for the Rexville strain. The precision of ID_{50} determination was no doubt enhanced by changing to a more infectious bloodmeal composition. The peroral ID_{50} for the Rockefeller strain was grossly estimated to be $7x10^9$ PFU/ml ingested, whan a less infectious artificial bloodmeal was used to infect the mosquitoes.

The monoclonal antibody (13A5.8) has been isolated in preparative quantities (ca. 2 gm) in a bioreactor. This antibody has been used to prepare mini-affinity columns for the isolation of WS2 and WR2 antigens from other mesenteronal brush border fragment (BBF) proteins. These two proteins are unique both in terms of their molecular sizes, 150 kD (WR2) and 100 kD (WS2), and their association with western equine encephalomyelitis (WEE) virus refractory (WR) and WEE virus susceptible (WS) strains of *Culex tarsalis*. The 13A5.8 antibody recognizes a common epitope on these two proteins (WR2 and WS2). This may suggest that through posttranslational modification (e.g., proteolytic cleavage) the nascent WR2 antigen is processed to the WS2 form.

In conjunction with the hypothetical relationship between WR2 and WS2, a smaller protein fragment (ca. 50-70 kD should be the produced and might be detectable in the WS2 BBF. A very unstable antibody (32G8) has recently been shown to have the required specificity to support the hypothesis. This antibody reacts with the nascent WR2 antigen but not to the WS2 antigen. Instead, the antibody reacts to a smaller fragment in the 50-70 kD range.

The 32G8 antibody may never be useful in further biochemical studies. The hybridoma is so unstable that it cannot be subcloned to increase viability or antibody production. It only yields diminishing numbers of antibody producing cells.

A cell culture model for alphaviral titer modulation was sought. Three lines of *Aedes albopictus* cells (U4.4, Singh and C6/36) cells were infected with WEE, venezuelan equine encephalomyelitis (VEE), Sindbis (SIN) and vesicular stomatitis (VS) viruses with an without the addition of the eucaryotic DNA dependent RNA polymerase inhibitor α-amanitin

(5 µg/ml). Both the U4.4 and Singh cell lines revealed a stimulation of WEE, VEE and SIN viral titers in treated cultures, while the C6/36 cell line was essentially uneffected. VS viral titers were also uneffected. It would appear that clones of either U4.4 or Singh cells could be selected that would provide a cell culture model for viral modulation.

Culex tarsalis females are known to modulate alphaviral titers through an unknown mechanism. A genetic line of nonmodulating (L.H) *Cx. tarsalis* was derived from modulaitng (LVP) *Cx. tarsalis*. The L.H line was derived through 13 generations of backcross matings and 6 brother-sister matings. These two lines should hypothetically differ by the single locus for modulation. They are congenic.

Parenteral inoculation reveals that the L.H line is not the genetically pure lined that we had anticipated. The genotype of this line should approximate 100% nonmodulating (HVP), however, 15% are still LVP. These are admittedly marginally LVP. The viral titers for this 15% within the L.H that modulate are minimally 3.6 $\log_{10}$ PFU/mosquito very near the cutoff point of ≥4.0 $\log_{10}$ PFU/mosquito for HVP. This margin of 0.4 $\log_{10}$ PFU/mosquito might very well be considered within the margin of error for viral titer determination by plaque assay in cell culture.

Peroral infection of three L.H lines reveals an ID_{50} essentially identical to the HVP strain of *Cx. tarsalis* (ca. 0.3-1.2 $\log_{10}$ PFU/ml ingested). The peroral ID_{50} for the LVP strain is >5.0 $\log_{10}$ PFU/ml ingested. After 10 days of extrinsic incubation, the percent infected for the L.H lines and HVP were virtually identical. However, after 14 days of extrinsic incubation, the percent infected for the L.H lines was significantly reduced. By definition, the congenic lines should only differ in the ability to modulate WEE virus. These results suggest that, at least in the selection of L.H from LVP that peroral susceptibility was also altered. These results suggest that further selection of the L.H lines might yield the desired genotypes.

Foreword

In conducting research using animals, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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II. Introduction

Whether a mosquito vector becomes infected with an arbovirus after feeding on a viremic vertebrate host depends upon a number of factors which are intrinsic to each individual mosquito within a population. The relative susceptibility of a vector population is most often referred to as vector competence. Many of the intrinsic factors which determine the vector competence of a mosquito population have been delineated and discussed with reference to the mosquito, *Culex tarsalis*, and the alphavirus, western equine encephalomyelitis (WEE) virus (Hardy et al., 1983).

Two model systems are available through which to examine the basis for vector competence in the *Culex* sp.-WEE virus system. The first to become available was an interspecific model: *Cx. tarsalis* and *Culex pipiens* (Houk et al., 1986). In this system, *Cx. tarsalis* is susceptible to WEE viral infection through both feeding and inoculation (i.e., bypassing mesenteronal infection). On the other hand, *Cx. pipiens* is highly refractory to feeding but susceptible to infection by inoculation. This situation has been referred to as the mesenteronal infection barrier (MIB). Specific receptor sites for WEE virions on mesenteronal epithelial cells would appear to be absent or functionally modified in *Cx. pipiens* and, thus, forms the basis for the MIB (Houk et al., 1986; Houk et al. In manuscript). An intraspecific mosquito model system has been developed by genetically selecting strains of *Cx. tarsalis* that are either highly susceptible (WS) or very refractory (WR) to WEE viral infection following feeding (Hardy et al., 1983; Kramer et al. 1989). The WR strain of*Cx. tarsalis* expresses two mechanisms that determine vector competence: absence of receptor sites and a mechanism that appears to control WEE viral replication (Hardy et al., 1983; Kramer et al., 1989; Ann. Prog. Rpt., 1988).

The ability of a mosquito to regulate the level and duration of detectable viral replication has been referred to as modulation (Murphy 1975; Murphy et al. 1975; Hardy et al. 1983; Kramer et al., 1989). We have recently concentrated our efforts on attempting to genetically isolate the genotype for viral modulation. We have low viral producing (LVP) and high viral producing (HVP and L.H) strains of the mosquito, *Cx. tarsalis*. The levels to which WEE virus multiplies in the refractory compared to susceptible strains differs by as much as 10,000-fold. It has been firmly established, through the use of DNA dependent RNA transcription inhibitors (i.e., actinomycin D and α-amanitin), that modulation is a property of the infected mosquito and not determined by the infecting virus (Houk et al., In manuscript).

A flavivirus-mosquito vector model similar to that available for the alphavirus-mosquito vector described above would be of interest. The literature (Murphy et al., 1975; Gubler et al., 1982) would suggest that such a model might be derived for dengue (DEN) virus and the mosquito, *Aedes aegypti*. Geographic strains of *Ae. aegypti* have been shown to vary significantly in their peroral susceptibility to DEN viral infection, suggesting that an MIB might be derived. While the literature does not reveal modulation of DEN viruses by mosquito vectors, the original observations of modulation occurred with flaviviruses, Japanese encephalitis (JE: Doi, et al., 1970) and St. Louis encephalitis (SLE; Murphy et al., 1975).

Herein, we report the progress made in the further characterization of the MIB and modulation of WEE viral titers in the mosquito, *Cx. tarsalis*. Progress toward the development of a flavivirus-mosquito model for MIB and modulation is also reported.

III. Mosquito Colonies Maintained for Vector Competence Studies

A number of mosquito species and strains that are relevant to our studies on the

TABLE 1. Mosquito colonies maintained for vector competence studies.

SPECIES	COLONY DESIGNATION
Aedes aegypti	Davis, Rexville, Rockefeller, Bangkok, Miami, Vero Beach
Aedes dorsalis	Ft. Baker
Culex peus	Grasshopper Slough
Culex pipiens	Poldervaart
Culex quinquefasciatus	Kern
Culex tarsalis	Chico; Ft. Collins; Knights Landing; Poso Creek; Manitoba; Yuma; WR-1 (FC-KL); WR-2 (FC-KL-C); WS-2 (KL); WS-3; LVP; L-HVP; HVP (KL); HVP (PC); HVP (Y)

vector competence of mosquitoes for both alpha- and flaviviruses are maintained in our insectary. In addition, a number of these strains represent unique genetic strains of *Cx. tarsalis* that are exemplary of barrriers associated with the inability to become infected with and the subsequent failure to transmit WEE virus.

During the past year, a number of geographic strains of *Ae. aegypti* have been examined for their susceptibility to peroral infection with DEN2 virus and, because of their extreme refractoriness, have been deleted. When a final decision is made with regard to the strain to be used for selection of the MIB model, the number of strains maintained in the insectary will be further reduced.

IV. Studies on the Vector Competence of *Aedes aegypti* for dengue viruses

The New Guinea C strain of DEN2 virus (kindly provided by Dr. S. Kliks, University of California, Berkeley CA) was selected for initial studies of peroral and parenteral infection of geographic strains of *Ae. aegypti* . Further, we have been able to obtain anti-dengue virus immune mouse ascitic fluid (Dr. E. Henchal, WRAIR, Washington DC) to allow detection of potential DEN viral infections by immunofluorescence assay (IFA) in mosquitoes and by *in situ* enzyme immunoassay (EIA) of infected cell cultures.

A. Developmental studies

1. *Determination of the efficacy of different feeding suspensions to infect mosquitoes with dengue 2 virus*

Two of the major problems associated with attempting to infect *Ae. aegypti* with DEN2 virus are the extreme peroral refractoriness of the mosquito to the virus and the relatively low titers to which the virus multiplies in cell culture. The literature contains many references to the presentation of artificial bloodmeals in a manner that results in more efficient feeding and/or infection of mosquitoes. We have compared a number of these methods in attempting to increase the efficiency of infecting the mosquito, *Ae. aegypti.*

In our last report (Ann. Prog. Rpt., 1988), we reported that the mosquito, *Ae. aegypti*, fed readily on droplets of blood placed directly upon the mesh of the holding cartons. This method allows a greater surface area for feeding, when compared to gauze pledgets, thus reducing competition between mosquitoes for access to feeding sites. The volume of blood

required to efficiently feed mosquitoes is significantly reduced by using the droplet method; 50-100 females may be easily fed on as little as 0.3-0.5 ml of infectious blood. All of our experiments use the droplet method in the infection of mosquitoes.

The production of an infectious bloodmeal with a titer sufficient to infect 100% of the mosquitoes fed is the goal of most investigators who wish to determine the comparative susceptiblity of geographic populations of mosquito vectors and the ID_{50} of any vector population for the virus being studied. The composition of the bloodmeal used to infect *Ae. aegypti* has been quite varied (Gubler and Rosen, 1976; Gubler et al., 1979; Miller et al., 1982; Miller, 1989; Watts et al., 1987). Since *Ae. aegypti* are very refractory to DEN2 virus, we elected to compare the effects of bloodmeal composition and the source of infectious DEN2 virus on the relative infectiousness of artificial bloodmeals.

We prepared infectious bloodmeals in several different ways according to published reports in the literature. Gubler and Rosen (1976) prepared feeding suspensions by combining equal proportions of frozen supernatant from infected C6/36 *Aedes albopictus* cell cultures, freshly washed red blood cells and 10% sucrose. Gubler et al. (1979) injected female mosquitoes with DEN2 virus and following 7 days of incubation at 28°C the mosquitoes were ground in 100% fetal calf serum. The mosquito suspension was clarified by centrifugation at 10,000xg-30 min and mixed with equal volumes of freshly washed red blood cells and 10% sucrose. Infected C/36 *Ae. albopictus* cell cultures were suspended by repeated pipetting, clarified by centrifugation (2000xg-10 min) and the cell pellet resuspended in 1.0 ml of the supernatant (Miller et al., 1982). This cell pellet suspension (0.1ml) was then mixed with freshly washed red blood cells (0.4 ml), fetal calf serum (0.45 ml) and sucrose (60% w/v; 0.5 ml). Another variation involved looking at the effect of freeze-thawing on the relative infectiousness of feeding suspensions made from infected cell culture supernatants (Gubler and Rosen, 1976; Miller, 1989). Feeding suspensions prepared from frozen stocks of DEN infected cell culture supernatants have been used to attempt to infect *Ae. aegypti* females prior to the studies reported herein (Ann. Prog. Rpt., 1988), but have not been compared to fresh DEN virus.

In this experiment (Fig. 1), we examine the infectiousness of DEN2 virus in several artificial bloodmeals of varying composition. Female mosquitoes (ca. 50/test) of the Rexville strain of *Ae. aegypti* were allowed to feed to engorgment, anesthetized with CO_2 and then held at 32°C for 11 days. After this extrinsic incubation period, mesenterons were dissected and viral infection determined by IFA (Kuberski and Rosen, 1977). The infectious bloodmeal

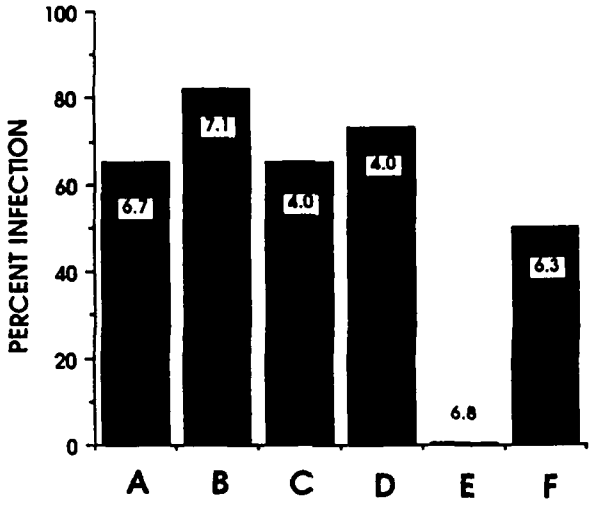

Figure 1. Comparison of the effects of composition on the relative infectiousness of dengue 2(New Guinea C) supplemented artificial bloodmeals. Bloodmeals were prepared according to Miller et al.[1983 (A, B, C, D)], Gubler et al. [1979 (E)] or Gubler and Rosen [1976 (F)]. A) Supernatant of infected BHK-21 cells, B) cell suspension of infected BHK-21 cells, C) supernatantof infected C6/36 cells, D) cell suspension of infected C6/36 cells, E) clarfied supernatant from inoculated mosquitoes homogenized in fetal calf serum and F) equal parts of infected C6/36 cell culture supernatant, freshlycollected red blood cells and sucrose (10%; w/v).

prepared from inoculated Rockefeller *Ae. aegypti* mosquitoes (E) could not be evaluated because only four mosquitoes survived to be assayed. However, an examination of the other artificial bloodmeals revealed extensive variation in infectiousness. Bloodmeals composed of infected cell pellet suspensions of either BHK-21 (D) or C6/36 (B) cells were able to infect 73% and 82% of the mosquitoes assayed, respectively. The cell culture supernatants were slightly less infectious; 65% of the surviving mosquitoes were infected when feeding on either BHK-21 (A) or C6/36 (C) supernatant bloodmeals. Only 50% of the mosquitoes that fed on the standard mixture (F) (Gubler and Rosen, 1976) became infected. The only difference between the standard mixture (F) and the C6/36 (A) supernatant bloodmeals was the fact that the standard mixture was made from DEN2 virus stocks that had been frozen. The viral titers of these two bloodmeals differed by only 0.4 $\log_{10}$ PFU/ml.

The reduction of viral titer as a consequence was of freezing was cause for concern, since it is extremely difficult to attain sufficiently high titered bloodmeals to routinely infect a high percentage of *Ae. aegypti* females by feeding. A routine examination of viral titers following freeze-thaw revealed that DEN2 was very susceptible to inactivation and suffered a loss of approximately 0.5 $\log_{10}$ PFU with each cycle (Data not shown). A similar observation was made by Miller (1989) in his study of the relative infectiousness of yellow fever (YF) virus presented as either freshly prepared or exposed to a single freeze-thaw cycle. Freshly prepared YF virus was significantly more infectious to mosquitoes, when compared to frozen virus. However, there was no difference in the infectiousness of YF virus prepared either fresh or following a single freeze-thaw cycle as assayed in BHK-21 cells. The loss of

infectiousness by mosquito feeding was correlated with a significant reduction in hemagglutination activity. Two things might be suggested: 1) the invertebrate cell receptor for YF virus may be related to the hemagglutination site on the virion and 2) viral receptors on BHK-21 cells and mosquito mesenteronal epithelial cells are structurally different.

2. *Centricon® filter concentration of cell culture supernatants as a means of increasing dengue 2 viral titers in artificial bloodmeals*

Since DEN2 viral titers in stocks grown in either vertebrate or invertebrate cell cultures were not high enough to infect 100% of any strain of *Ae. aegypti* evaluated, we sought a rapid, gentle method to increase viral titers in artificial bloodmeals. Centricon-30® filters are integral plastic devices with a 30,000 molecular weight dialysis membrane rigidly supported by a plastic grid to allow centrifugation at moderate g-forces to facilitate removal of lower molecular weight materials. This means of sample concentration seemed well suited to our studies of DEN2 virus because the entire concentration process can be accomplished within 1 hour and the virus is exposed to an environment of increasing protection through concentration of high molecular weight proteins added with fetal calf serum without increasing the concentration of low molecular weight salts.

TABLE 2. Determination of the concentration factor for Centricon-30® filters through comparison of volume reduction and dengue 2 (New Guinea C) viral titer increase.

Experiment	Concentration factor	
	Volume[1]	Viral titer[2]
1	19	20
2	12	10
3	12	10

[1] The volumetric concentration factor was determined by comparing the volume of supernatant before and after centrifugation at 3500xg-30 min.

[2] The viral titer concentration factor was determined by comparing the viral titer in the supernatant before and after centrifugation at 3500xg-30 min.

Several experiments attempted to correlate the observed volume concentration of cell culture supernatants with viral titer increases in an attempt to determine the efficacy of this

method. In other words, can one observe similar concentration factors in both volume of cell culture supernatants and DEN2 viral titers (Table 2). It would appear that the answer is unequivocally affirmative.

B. Characterization of geographic strains of Aedes aegypti for their vector competence with dengue viruses - Variations in peroral and parenteral susceptibility

We continued our assessment of the vector competence of various geographic populations of *Ae. aegypti* for DEN2 virus. Many of the populations examined were too refractory to peroral infection and were eliminated as candidates for selection of a MIB model for the flaviviruses. Therefore, we subsequent studies on mosquito peroral susceptibility utilized the Rexville and Rockefeller strains. The Rexville strain showed a consistent 30+% susceptibility at the highest viral challenge doses and was a vigorous, long-lived strain. The Rockefeller strain continues to be a candidate strain because it is perhaps the most susceptible strain we have examined to date and its genetics are well documented in the literature. A significant drawback, however, is the rather inconsistent survival of adult females of both strains during the extrinsic incubation period of 11 days at 32°C, which has been shown to be the minimal incubation period for DEN2 virus in mosquito vectors at this temperature (Watts et al., 1987).

We chose to continue our assessment of infection through plaque assay of whole mosquito homogenates rather than to continue IFA for detection of viral antigens in dissected mesenterons. Plaque assay is very time consuming, protracted assay but the results are definitive. The IFA was plagued with a number of problems. The failure of some dissected mesenterons to adhere to the glass slide during processing for staining decreased the sample size. In many experiments, the attrition of females during the 11 days of extrinsic incubation was significant; thus, it was imperative that all surviving mosquitoes be assayed without further reduction in numbers. In addition, the IFA gave questionable results for viral infection of some mesenterons. The use of the plaque assay essentially eliminated these two major problems associated with the use of IFA to assess DEN2 viral infections in mosquitoes.

Mosquito mortality during the 11 days of extrinsic incubation at 32°C is a consistent problem with each experiment. However, the strain of mosquito that experiences excessive mortality in any particular experiment is not consistent. We have no explanation for this mortality except that this is an unusually high temperature for most mosquitoes. In one peroral infection experiment, as an adjunct, we attempted to determine if there was any

suggestion of a relationship between mosquito mortality and DEN2 viral infection. The suggestion of a relationship between these two parameters would lead to an experiment designed to closely follow mortality and infection during the period of extrinsic incubation. Each day of this experiment, mosquito holding cartons were examined and all dead or moribund mosquitoes removed and frozen at -70°C until assay for virus (Table 3). The mosquitoes had been allowed to feed on Centricon® concentrated DEN2 viral suspensions [C] and DEN2 viral infected C6/36 cell suspensions, resuspended in Centricon® concentrated infectious supernatant [CS/C]. No mortality occurred until day 9 at which time in the [CS/C] groups 4 (20%) Rockefeller and 1 (4%) Rexville were frozen for assay. Of the [C] groups, 7 (20%) Rockefeller and 1 (6%) Rexville were frozen for assay. No mosquitoes were found to have died during the period between day 9 collection and examination on day 10. On day 11 in the [CS/C] groups, 3 (16%) Rockefeller and 7 (28%) Rexville were frozen for assay. In the [C] groups, 17 (48%) Rockefeller and 5 (12%) Rexville were frozen. Total mortality during the experiment for those mosquitoes fed on [CS/C] was 37% for Rockefeller and 25% for Rexville and for those fed on [C] 69% for Rockefeller and 15% for Rexville.

TABLE 3. An examination of infection rates and mortality in the Rockefeller and Rexville strains of the mosquito, *Aedes aegypti*, following peroral infection with dengue 2 (New Guinea C) virus.

Type of Infectious Bloodmeal	Non-surviving mosquitoes Percent mortality(Percent infection) 9 days	11 days	Surviving mosquitoes Number tested(Percent infection) 11 days
[CS/C]*			
Rockefeller	20(25)	16(33)	12(58)
Rexville	4(0)	28(14)	24(21)
[C]*			
Rockefeller	20(14)	48(12)	11(18)
Rexville	2(0)	13(0)	20(5)

* CS/C=3500xg-30 min cell pellet from an infected C6/36 cell culture resuspended in cell culture supernatant that had been concentrated approximately 10-fold in a Centricon® concentrator. C=C6/36 cell culture supernatant clarified by centrifugation at 3500xg-30 min and then concentrated approximately 10-fold in a Centricon® concentrator.

The infection rates of infection were determined for dead and moribund mosquitoes collected on days 9 and 11 of extrinsic incubation and compared to the infection rates for those mosquitoes that survived the 11 days of incubation. In all cases examined, the infection rate was higher in mosquitoes surviving until day ll of extrinsic incubation than in those dead or moribund mosquitoes collected on days 9 and 11 of extrinsic incubation (Table 3). There would not appear to be any suggestion of a relationship between mortality prior to termination of extrinsic incubation at 11 days at 32°C and DEN2 viral infection.

The extreme refractoriness of all geographic strains of *Ae. aegypti* examined to date presents a real problem in the pursuit of a MIB model for flaviviruses. Miller and Ballinger (1989) and Miller (1989) reported that *Ae. aegypti* were more susceptible when fed on infectious bloodmeals comprised of resuspended infected cell pellets, compared to infectious supernatants. The relative infectiousness of three different variations on this theme were examined (Table 4). The major variation was an attempt to increase infectious viral titers through the use of Centricon-30® filters (See section IV.A.2). These filters are capable of increasing the viral titers of cell culture supernatants by 10-fold or more; advertised up to

TABLE 4. Comparison of the peroral susceptibility of the Rockefeller and Rexville strains of *Aedes aegypti* fed on infectious bloodmeals of differing composition.

Bloodmeal	Viral titer (PFU/ml)	Rexville Percent infected (Number tested)	Rockefeller Percent infected (Number tested)
Experiment 1			
C*	$1.0x10^8$	5(22)	0(5)
CS	$3.5x10^7$	0(19)	0(9)
CS/C	$1.0x10^8$	17(28)	33(3)
Experiment 2			
C	$5.0x10^8$	0(10)	0(7)
CS	$5.0x10^7$	0(8)	50(6)
CS/C	$2.0x10^8$	22(9)	35(20)

* C=Centricon-30® concentrated, dengue 2 infectious C6/36 supernatant. CS=3500xg-30 min, dengue 2 infected, C6/36 cell pellets resuspended in 1 ml of unconcentrated, dengue 2 infectious C6/36 supernatant. CS/C=CS resuspended in 1 ml of C. The composition of the artificial bloodmeal was 0.1 ml infectious material (C, CS, or CS/C), 0.4 ml freshly washed red blood cells, 0.45 ml fetal calf serum and 0.05 ml sucrose (60%; w/v).

40-fold. However, the increase in supernatant viral titer apparently does not increase the relative infectiousness of the bloodmeals containing no cell suspension (C), only 1 mosquito of the combined 44 Rexville and Rockefeller mosquitoes tested was positive (Table 4). Only 3 of the combined 43 mosquitoes that fed on the CS bloodmeal became infected. All 3 of these mosquitoes were Rockefeller from experiment 2. However, when one combines CS and C (i.e., CS/C), 25% of the combined mosquitoes (15/60) were positive for DEN2 viral infection. It would appear that, of the bloodmeal compositions examined, a bloodmeal containing suspensions of infected C6/36 cells resuspended in Centricon-30® concentrated infectious supernatant is the most infectious for *Ae. aegypti*. This corroborates the observations reported above (Fig. 1).

By combining the data from three separate experiments, a crude estimate of the peroral ID_{50} to infect the Rockefeller and Rexville strains was obtained (Fig. 2). Of the two strains, the Rockefeller strain was somewhat more susceptible with an ID_{50} of $8x10^8$ PFU/ml ingested (Fig. 2A), compared to an estimated ID_{50} of approximately $1.7x10^9$ PFU/ml ingested

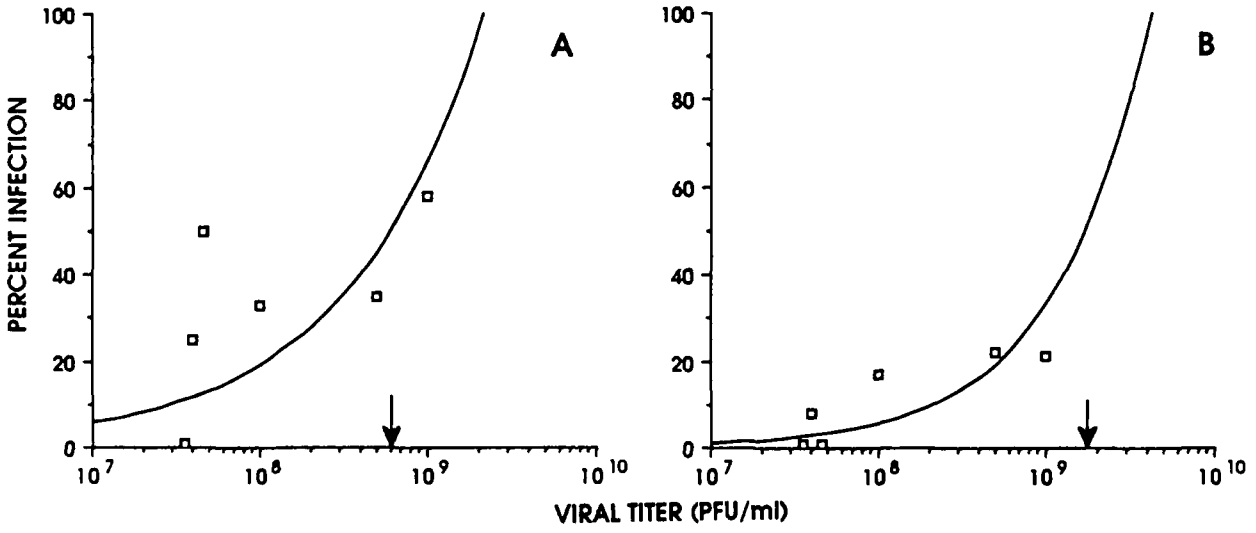

Figure 2. Graphic determination of the apparent ID_{50} following peroral infection ofthe Rockefeller (A) and Rexville (B) strains of *Aedes aegypti* with dengue 2 (New Guinea C) virus and 11 days of extrinsic incubation. Artificial bloodmeal was prepared from infected C6/36cells as described by Miller etal. (1983).

for the Rexville strain (Fig. 2B). In the 1988 Annual Progress Report, we reported a peroral ID_{50} for the Rockefeller at approximately $7x10^9$ PFU/ml ingested. This difference, almost 10-fold, is most likely related to an alteration in the artificial bloodmeal composition between the two years (Fig. 1). In the previous study (Ann. Prog. Rpt., 1988), we attempted to infect mosquitoes with a mixture of equal parts of red blood cells, sucrose (10% w/v) and cell

culture supernatant; standard mixture (Fig. 1). In our present studies, mosquitoes were fed on a suspension of red blood cells, fetal calf serum, sucrose and resuspended cells from infected C6/36 *Ae. albopictus* cell cultures (Fig. 1).

A number of future experiments are suggested based on the results of the many and varied attempts to infect *Ae. aegypti* perorally. First, the genetic selection for the MIB will be initiated using the Rockefeller strain. We should be able to routinely infect 30-50% of this strain with our highest titered cell suspensions. This should provide an adequate starting point to derive, following several generations of selection, a more susceptible strain of *Ae. aegypti*. Second, the apparently high infectiousness of BHK-21 derived virus, as compared to C6/36 derived virus, will be further investigated (Fig. 1). BHK-21 virus will be blind passaged several times to determine if viral replication (titers) can be enhanced (increased). Third, the large discrepancy in the percent of the Rexville strain infected between the experiment assayed at 7 days by IFA (Fig. 1) and those assayed at 11 days by plaque assay (Table 4) should be further examined. A reduction in the percent infected mosquitoes with time is indicative of the modulation of viral titer by the mosquito vector. A carefully monitored experiment must be completed to attempt to verify that modulation of DEN2 viral titers occurs in *Ae. aegypti*. However, affirmation of this observation would not be too surprising since modulation was first reported in flaviviruses (Doi, 1970; Whitfield et al., 1971; Whitfield et al., 1973; Murphy et al., 1975).

V. Vector Competence Studies on *Culex tarsalis* and Western Equine Encephalomyelitis Virus

A. Mesenteronal infection barrier

1. *Monoclonal antibodies to brush border fragment proteins*

Several years ago we undertook the problem of producing monoclonal antibodies to the mesenteronal brush border proteins of susceptible and refractory *Cx. tarsalis* (Final Rpt., 1987; Ann. Prog. Rpt., 1988). The original concept was that by producing antibodies unique to proteins of susceptible versus refractory mosquito mesenterons that a specific receptor protein for WEE virus might be identified and/or isolated. The impetus for this study was two significant observations: 1) peroral refractoriness in mosquitoes is related to the failure of ingested virus to infect mesenteronal epithelial cells (i.e. a MIB) and 2) brush border proteins

from *Cx. tarsalis* mesenterons, differ significantly when susceptible (WS2) and refractory (WR2) mesenterons are compared (Fig. 3).

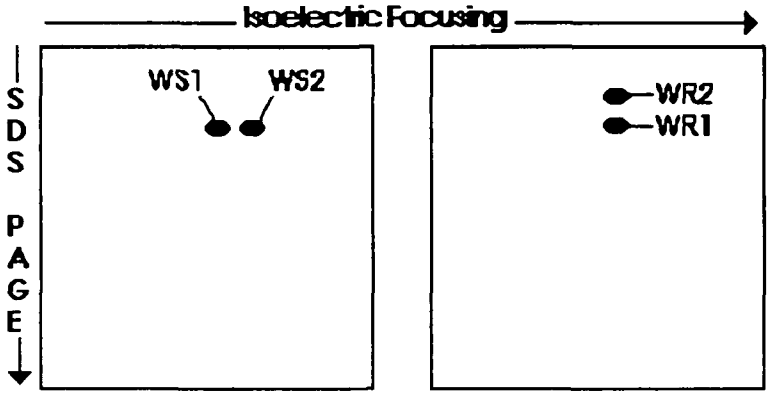

Figure 3. Schematic representation of the two dimensional (2D) electorphoretic separation of the two major proteins found in mesenteronal epithelial cell brush border fragments. The molecular weights of the proteins are about 150 kD for WR2 and 100 kD for WR1, WS1 and WS2..

A number of unique monoclonal antibodies were identified in our initial screening of hybridomas, prior to our move from the Naval Biosciences Laboratory (Ann. Prog. Rpt., 1988). However, none of these hybridomas were viable upon reculture and expansion in cell culture following the move. We have been able to examine a number of hybridomas from our initial fusions that are cross reactive between susceptible and refractory mesenterons. Two of these, 13A5 and 13C1, have been subcloned twice. The 13C1 monoclonal is an IgM and, thus, has restricted utility. The 13A5 monoclonal antibody is an IgG_{2b} of very high affinity. The 13A5 antibody, as mouse immune ascitic fluid, gives a good reaction with native blotted antigen (Reinhart and Malamud, 1982) at dilutions as high as 1:16,000 (Fig. 4). The antibody only reacts to the native antigen; no reaction against SDS denatured, western blotted antigen (Towbin et al., 1979). The 13A5 antibody appears to be specific to a conserved epitope on the protein that is differentially expressed in susceptible (WS2) and refractory (WR2) mesenterons.

Mass production of one of the subclones of the 13A5 monoclonal antibody (13A5.8) was undertaken by the Hybridoma Laboratory (Dr. Alex Karu), as part of a study to determine the feasibility of the production of antibodies in bioreactors. Two important benefits of this type of approach to the mass production of antibodies are: 1) the rapidity with which one can generate gram quantities of antibody (ca. 30 days) and 2) there are no

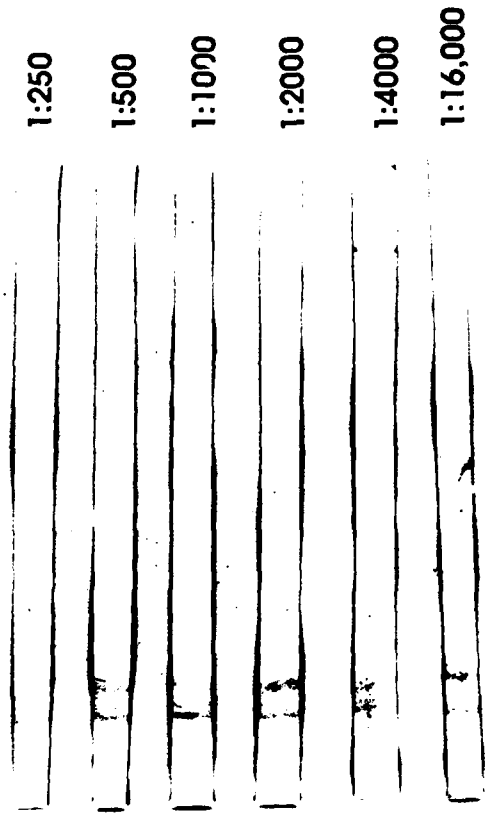

Figure 4. Effect of the dilution of 13A5.8 monoclonal antibody ascites fluid on the detection of brush border antigens from native blotted isoelectric focused electrophoretic gels.

requirements for animals. One major disadvantage is the very high cost of producing antibody, compared to the production of mouse immune ascitic fluid. However, since this was a pilot study, our antibody, which is 1-2 grams ammonium sulfate precipitated protein, was produced gratis. This antibody preparation awaits protein A column purification.

Two major interests are linked to the 13A5 antibody and its apparent specificity for the major proteins that are variant in refractory and susceptible *Cx. tarsalis* mesenteronal brush borders. First, the antibody can be used to isolate the WS2 protein from crude preparations of susceptible mesenterons through the use of affinity columns. This particular protein is very difficult to separate from the other major protein in mesenteronal brush borders, WS1, even by 2D electrophoresis (Fig. 3). The isolated protein, along with its counterpart from refractory mesenterons (WR2), can now be examined by peptide mapping and microsequencing procedures to confirm, or refute, the contention that these proteins are related and their differences in molecular weight are related to a failure in posttranslational

cleavage of the protein in refractory mosquitoes. Second, and perhaps more importantly, this antibody is specific to the two proteins that are obviously variant in susceptible and refractory *Cx. tarsalis*. One might hypothesize that the WS2 protein is the receptor for WEE virus and that the WR2 protein, because of problems with posttranslational modification, represents a dysfunctional receptor. The experiment to attempt to block the attachment of WEE virus to brush border fragments of susceptible *Cx. tarsalis* with 13A5 antibodies will be undertaken in the immediate future. Even if 13A5 does not block attachment, this antibody can allow us to isolate large quantities of WS2 and WR2 antigens. These antigens can be used to produce polyclonal antibodies that would allow us to simultaneously screen a number of epitopes on these antigens to determine if this protein is the specific receptor for WEE virus.

Two small-scale (1 ml) affinity minicolumns were prepared with 13A5.8 monoclonal antibody that had been purified by protein A affinity chromatography (Ann. Prog. Rpt., 1988). The column affinity matrixes were Affi-gel® (BioRad Laboratories) and AminoLink® (Pierce Chemicals). Preliminary runs on these columns revealed that the Affi-gel® column either did not retain (i.e., covalently link) very much of the antibody or there was a significant loss in affinity between the antigen and antibody following covalent linkage. However, the AminoLink® column bound the antibody quite efficiently. Brush border fragments (WS) were radiolabeled with ^{3}H-borohydride (Schaffer and Siegel, 1976), solubilized in Tris (0.2M; pH 6.7) -Triton X-100 (1%; v/v) and the radiolabeled brush border proteins allowed to bind to the AminoLink® column for 1 hr. The column was washed with PBS (ca. 10 ml) to

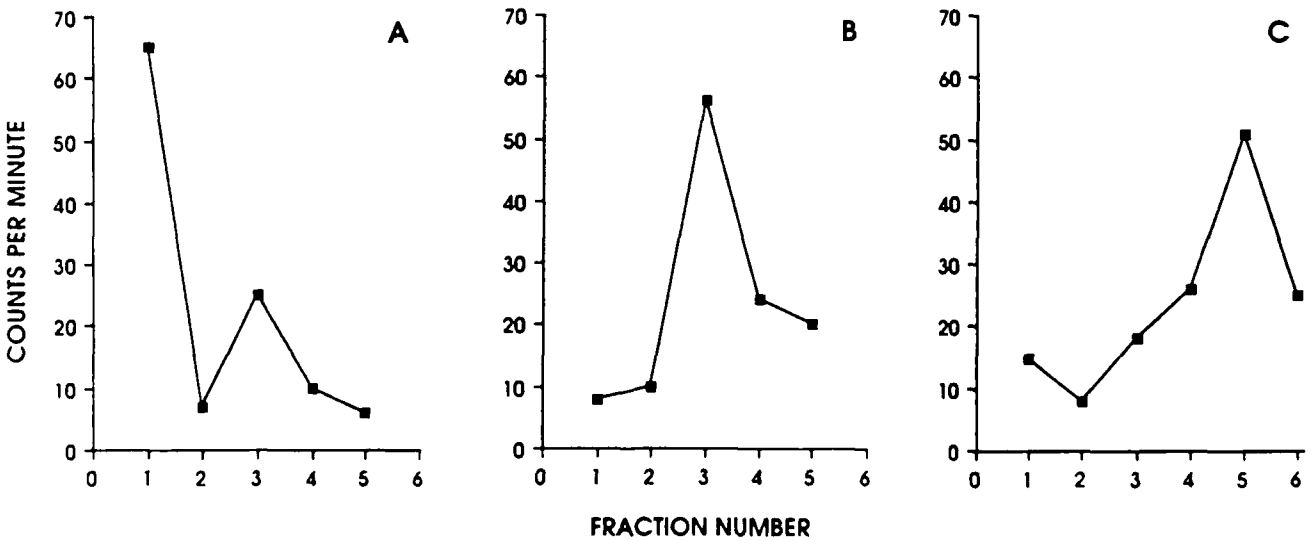

Figure 5. Elution profiles of ^{3}H-radiolabeled brush border proteins bound to 13A5.8 antibody minicolumns. A) WS brush border proteins on AminoLink®, B) WR brush border proteins on AminoLink® and C) rechromatography of the flow-thru from A and B on an Affi-Gel® column.

remove unbound proteins. Protein(s) presumably bound to 13A5 antibodies was eluted as 1 m' fractions with 0.1 M glycine (pH 2.8). The fractions were monitored by counting a 100 µl aliquot of each fraction (Fig. 5A). Following elution of the bound WS proteins, WR proteins were bound to and eluted from the AminoLink® column under identical conditions (Fig. 5B).

The unbound fractions from the AminoLink® column of both the WS and WR proteins were applied to the Affi-Gel® column to determine if any further proteins (i.e., WS2 and WR2) could be isolated. A peak of radioactivity did elute from this column but the peak faction was delayed by 2 ml when compared to the AminoLink® column (Fig. 5C).

The peak fractions from each of the three column purifications were precipitated with trichloroacetic acid (10%) for 1 hr at 5°C, precipitates collected by centrifugation for 15 min in a microfuge, and the supernatants decanted. The pellets were allowed to air dry overnight, solubilized in 20 µl of SDS sample buffer and analyzed by SDS-PAGE. The fraction from WS

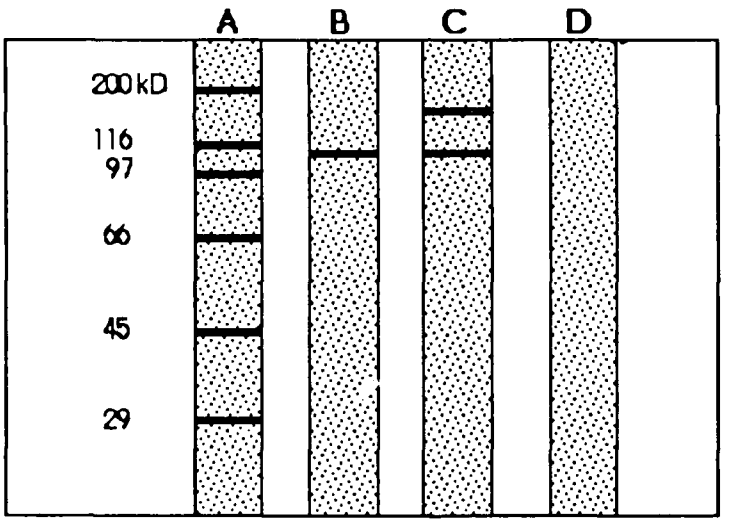

Figure 6. Schematic of the SDS-PAGE, Coomassie blue stained electropherogram of the eluted peaks from 13A5 monoclonal antibody affinity columns; B and C on AminoLink® and D on Affi-Gel®. A) Molecular weight standards, B) WS brush border protein, C) WR brush border proteins and D) WS and WR flow-thru from Amino-Link® column rechromatographed on Affi-Gel® column.

proteins contained a single protein of the appropriate molecular weight for WS2. The WR proteins were not so clear-cut. There were 2 proteins visible; one at the appropriate molecular weight for WR2 (ca. 150 kD) but another protein at 100 kD. This latter could have been either WR1 protein with an affinity for the 13A5 antibody or, most likely, WS2 protein

that did not elute in the 6 mls of elution buffer used for the first elution. Although there was a peak of radioactivity from the Affi-Gel® column (Fig. 5C), no proteins were observed in the Coomassie blue stained gel. An attempt to enhance the sensitivity of protein detection in the gel through silver staining destroyed the gel. Thus, no photograph is available but a schematic of the results is presented (Fig. 6). However, subsequent to the date of this report, we have eluted more WS2 and WR2 proteins from the 13A5 antibody mini-column. Again, there was evidence of a contaminating(?) band in both the WR and WS peaks. This problem may be alleviated by preparing fresh AminoLink® columns; one for each strain of mosquito (protein) to be examined.

2. *New monoclonal antibodies to brush border proteins*

As discussed in previous reports (Ann. Prog. Rpt., 1988) and above (V.A.1), the hybridomas producing antibodies unique to brush border proteins from susceptible or refractory mosquitoes did not survive the transition from the Naval Biosciences Laboratory to the Berkeley Campus. As a consequence, the production of a new panel of hybridomas was undertaken. Acrylamide gel fragments were masceratаed in Freund's incomplete adjuvent

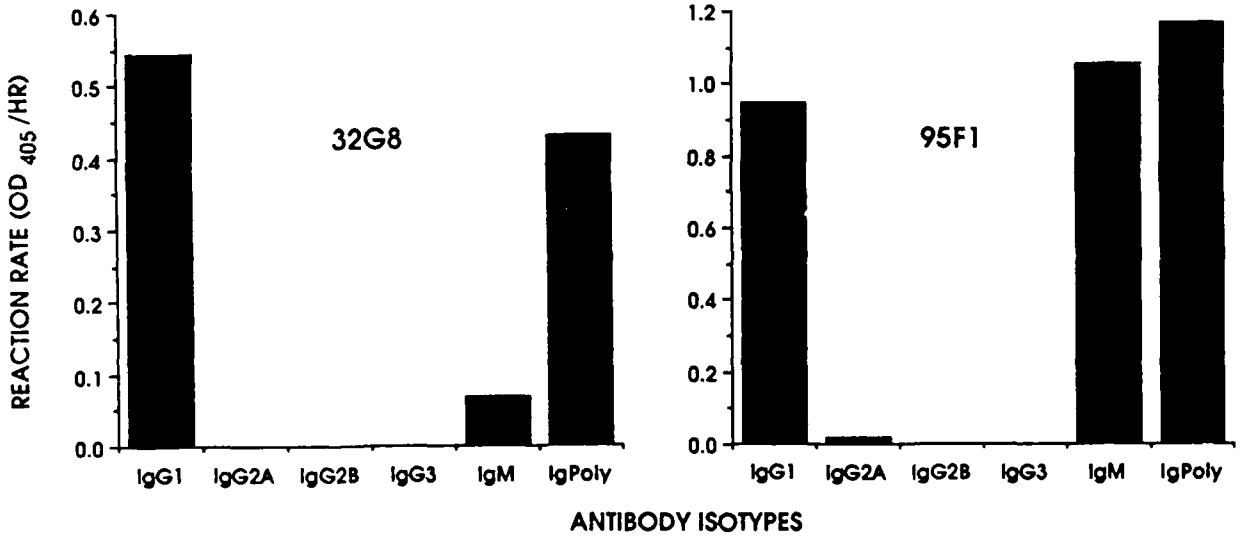

Figure 7. Antibody isotype determination for 32G8 and 95F1 hybridomas.

and inoculated into mice subcutaneously. Sleens were surgically removed and fusions with myeloma cells attempted. These fusions were minimally successful. One anticipated approximately 1000 hybridomas would be produced, instead 160 hybridomas were screened.

Of these 160 hybridomas, only 2 produced antibody to brush border proteins at detectable levels; 32G8 and 95F1.

The hybridomas, 32G8 and 95F1, are very unstable and do not produce large quantities of antibody. They are so unstable that even as they were being subcloned only a small number of the clones continued to produce antibodies. These antibodies react to epitopes on the native protein as determined by enzyme linked immunoassay and to denatured proteins as determined by western blotting of SDS-PAGE gels. The isotypes of these antibodies are IgG_1 for 32G8 and a mixed producer of IgG_1 and IgM for 95F1 (Fig. 7).

The 32G8 monoclonal antibody is rather unique in its reactivity to western blotted brush border proteins of susceptible and refractory *Cx. tarsalis* (Fig. 8). This antibody reacts to an antigen in the region of the WR2 protein (ca. 150 kD). The reaction in WS is against a

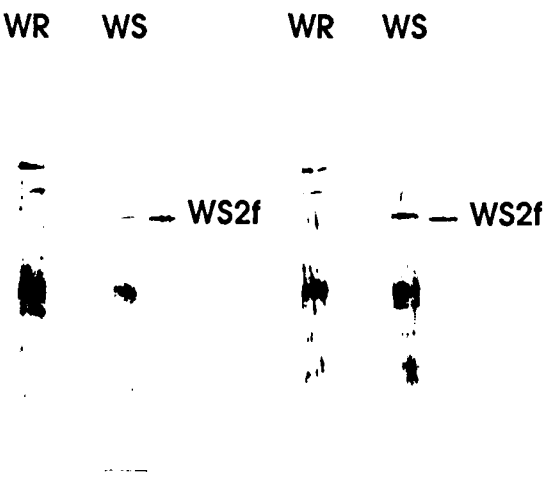

Figure 8. Western blot of the reactivity of 32G8 hybridoma supernatant antibody against WR and WS antigens. Note the lower molecular weight fragment (WS2f) detected in the 50-70 kD range in the WS blots.

protein in the 50-70 kD range. This pattern of reactivity is supportive of an earlier hypothesis, repeated many times in previous progress reports, to explain the relationship between the two proteins, WS2 and WR2 (Fig. 9). It has been suggested that in the normal processing of a nascent protein, perhaps equivalent to WR2, that a proeolytic posttranslational event occurs that results in the WS2 protein and a smaller unidentified fragment. One of our original thoughts was that is we could generate monoclonal antibodies to the WR2 protein that perhaps an epitope on this protein might survive the

posttranslational modification and be identified as a smaller fragment in western blots of WS brush border proteins. The 32G8 monoclonal antibody may have identified this fragment. Further experiments, especially microsequencing, may well answer this and other questions with regard to the relationship between WS2 and WR2 proteins. However, the instability of the 32G8 hybridomas may preclude their use in the identification (differentiation) of these proteins.

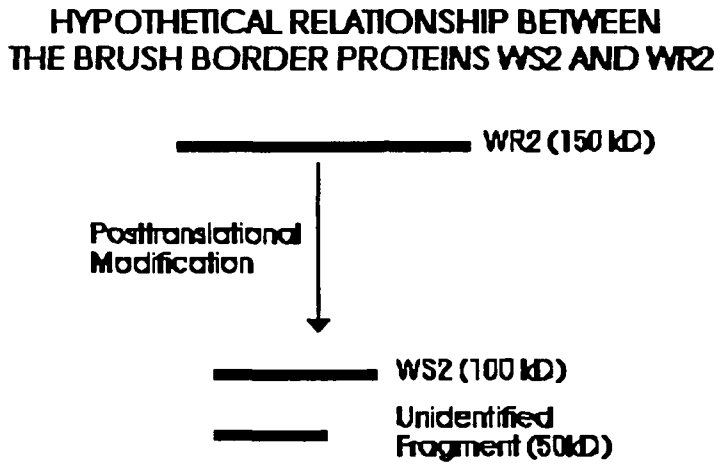

Figure 9. Schematic representation of the presumed relationship between two of the major proteins found in the brush border fragments of WS and WR *Culex tarsalis*.

A new inoculation sequence, using antigen electroeluted from SDS-PAGE gels, has been scheduled. This new scheme was suggested after a single mouse, previously immunized with SDS-PAGE gel fragments, received a booster inoculation of electroeluted antigen. The mouse responded with an extremely high antibody titer.

SDS-PAGE of brush border proteins allows one to cleanly separate of WR1 and WR2 proteins but does not separate WS1 and WS2 proteins. Isoelectric focusing electrophoresis doesn't allow the clean separation of either of the protein pairs, although resolution of WS1 and WS2 is often possible (Fig. 3). We have chosen to use SDS-PAGE as the method for the production of antigen for mouse immunization. We will be able to separate the two WR proteins and immunize mice with the individual proteins. The WS proteins will be used together as an immunogen, since their molecular sizes are identical. It is most important that WR2 be used as a clean immunogen if we hope to identify the hypothetical protein fragment from posttranslational modification (Fig. 9). In addition, depending on the progress in the

separation of WS1 and WS2 antigens through antibody-affinity chromatography (see V.A.1), the WS mice may receive booster inoculations comprised of isolated WS1 or WS2 antigen.

B. Modulation of viral titers in mosquitoes and mosquito cell cultures

1. *The effects of eucaryotic transcription inhibitors on viral titers in* Aedes albopictus *cell cultures*

Tooker and Kennedy (1981) reported the selection of approximately 100 clones of *Aedes albopictus* (Singh). These clones produced widely variant titer of Semliki Forest virus following infection with identical concentrations of virus. This observation is quite reminescent of our initial observations of mosquito populations that also expressed a very wide range of WEE viral titers when individual mosquitoes were examined after a suitable period of extrinsic incubation. These early observations led to the genetic selection of low virus producing (LVP) and high virus producing (HVP) strains of the mosquito, *Cx. tarsalis*. It has been suggested that the basis for modulation resides with the mosquito and not the viral strain (Murphy, 1975; Murphy et al., 1975; Hardy et al., 1983; Ann. Prog. Rpt., 1987). The LVP and HVP strains have been further characterized by determining the effects of transcription inhibitors [i.e., actinomycin D (actD) and α-amanitin (αA)]. In these studies (Ann. Prog. Rpt., 1987; 1988; Houk et al., In manuscript), it has been demonstrated that the effects of the transcription inhibitors are to allow WEE virus to multiply to much higher titers in the LVP mosquitoes, while only slightly elevating the titers in HVP mosquitoes. It would appear that LVP mosquitoes produce a factor that modulates the level to which WEE can multiply.

The problem of viral titer modulation could be addressed readily through the use of subtractive cross-hybridization of cDNAs synthesized from the mRNA populations isolated from LVP and HVP mosquito populations. Two approaches to this problem are: 1) the selection of congenic lines of mosquitoes that hypothetically differ only in the single locus of interest (see section V.B.2) and 2) the production of cell culture clones that can be used to produce a model viral modulation system.

Cell cultures of *Ae. albopictus* are available that differ significantly in their ability to support the multiplication of Sindbis virus (Condreay and Brown, 1989); a low virus producing line (U4.4) and a high virus producing line (C6/36). The two cloned cell lines and the uncloned cell line (Singh) were acquired and the effects of the transcription inhibitor αA on

viral titers in each cell line determined. The viruses examined were Sindbis (SIN), Venezuelan equine encephalomyelitis (VEE), WEE and vesicular stomatitis (VS) (Fig. 10). The effects were rather astounding in their magnitude of response but not unlike the response of LP and HP mosquitoes for WEE virus. U4.4 and Singh cells both revealed significant

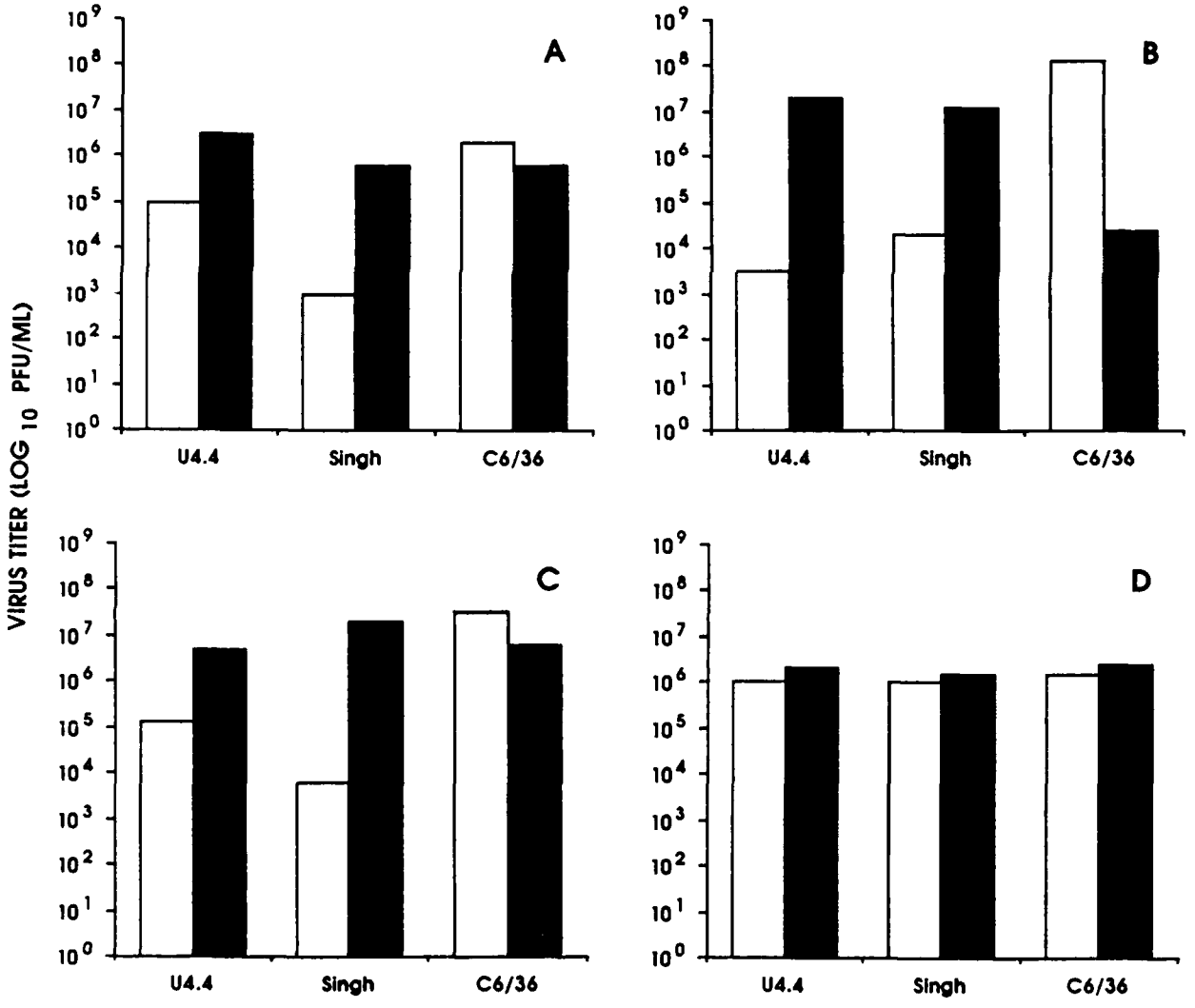

Figure 10. The effects of α-amanitin (5 μg/ml) on the titers of three alphaviruses, Sindbis(A), western equine encephalomyelitis (B), Venezuelan equine encephalomyelitis (C), and the nonalphavirus, vesicular stomatitis virus (D)in three strains of *Aedes albopictus*cells: U4.4, Singh and C6/36.

increases in alphaviral titers (VEE, WEE and SIN) when treated with αA (5 μg/ml) (Fig. 10A, B, C). Increases in viral titer were between a minimum of about 2.0 $\log_{10}$ PFU/ml to a maximum of approximately 4.0 $\log_{10}$ PFU/ml. Sin and VEE viral titers were only slightly effected in C6/36 cells (Fig. 10A, C). In contrast, WEE viral titers were drastically reduced

when C6/36 cells were treated with αA (Fig. 10B). VS viral titers were not as significantly effected by αA treatment as those of the alphaviruses (Fig. 10D). A 0.5 $\log_{10}$ PFU/ml increase in VS viral titer in U4.4 cells was the maximum response.

2. *Comparison of the peroral and parenteral susceptibilities of the congenic lines (LVPand L.H) of* Culex tarsalis.

LVP and HVP strains of *Cx. tarsalis* differ markedly in their ability to modulate WEE viral titers (Ann. Prog. Rpt., 1983). However, these two strains also differ in innumerable other genetic traits. It has been suggested that subtractive cross-hybridization studies be undertaken to attempt to isolate specific mRNA species related to modulation. Since, a number of other genetic traits vary between LVP and HVP strains, it was suggested that congenic lines of these mosquitoes be genetically selected to minimize genetic variability (J.H. and E.G. Strauss, Pers. Commun). Theoretically the two congenic strains should differ at only one genetic locus; the one selected. The Annual Progress Report (1988) outlined the genetic selection scheme. The parental LVP strain of *Cx. tarsalis* also expresses some (10-15%) of the HVP phenotype (i.e., produces $\geq 10^4$ PFU/mosquito). Following the congenic selection scheme, the HVP phenotype was theoretically supposed to be expressed in 100% of the progeny. This new strain (L.H) is theoretically identical to LVP except that it does not modulate inoculated WEE virus but allows the virus to grow to high titers (i.e., congenic).

The peroral and parenteral susceptibilities of these mosquitoes were examined to determine whether these mosquitoes were similar to the parental strain in these two important charactaeristics. A comparison of the parenteral infection results for LVP, L.H and HVP is presented in Table 5. The LVP used in this experiment reveal the same proportion of HVP as the original population of LVP from which the L.H was derived starting 2 years ago. The HVP line remains 100% HVP with no viral titers below 6.4 $\log_{10}$ PFU/mosquito; 700-fold more virus than the minimum required to be classified as HVP. The congenic line (L.H) still retained some LVP characteristic, in that 3 individuals tested had viral titers below 4.0 $\log_{10}$ PFU/mosquito. The level to which the virus replicated in these individuals may well be within the error of viral titration by plaque assay. However, the fact remains that there are some individuals within the population that apparently continue to express modulation. This is a disappointing observation.

The results for peroral infection were initially encouraging but these results also indicate that our congenic lines are somewhat tarnished (Table 6). The peroral susceptibilities of

three congenic lines (i.e., L.HT, L.HM and L.HX) were compared to LVP and HVP. The LVP remained very refractory and the HVP were highly susceptible. In terms of peroral susceptibility, the congenic lines were HVP in phenotype with a range of ID_{50}'s from

TABLE 5. The comparative susceptibility of low virus producing (LVP), high virus producing (HVP) and congenic (L.H) strains of the mosquito, *Culex tarsalis*, to parenteral infection with western equine encephalomyelitis virus (BFS 1703).

Mosquito Strain	Doseage[1]	Viral titer[2] Mean (Range)	LVP:HVP[3]
LVP	2.3	2.7(≤1.3-5.0)	18: 3
L.H	1.3	6.1 (3.6-7.0)	3:28
HVP	1.3	6.3 (6.4-7.0)	0:29

[1] Log_{10} PFU of WEE virus (BFS 1703, SMp2) inoculated intrathoracically per mosquito.
[2] PFU/ mosquito as determined by plaque assay in Vero cells.
[3] The number of positive mosquitoes that had titers of <4.0 log_{10} PFU (LVP) versus those that had titers of ≥4.0 log_{10} PFU (HVP).

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TABLE 6. The comparative susceptibility of low virus producing (LVP), high virus producing (HVP) and three congenic (L.HT, L.HM, L.HX) strains of the mosquito, *Culex tarsalis*, to peroral infection with western equine encephalomyelitis virus (BFS 1703).

Mosquito strain	Extrinsic incubation (Days)	$\log_{10}$ PFU virus ingested per mosquito -1.2	-0.2	1.7	2.9	4.6	ID_{50}
LVP	10	NT[1]	NT	0(20)[2]	5(20)	16(19)	>5.0
L.HT	10	20(20)	45(20)	55(20)	90(20)	100(20)	0.3
	14	NT	NT	NT	40(20)	NT	
L.HM	10	15(20)	60(20)	60(20)	90(20)	100(20)	0.1
	14	NT	NT	0(5)	40(15)	NT	
L.HX	10	5(20)	30(20)	55(20)	90(20)	100(20)	1.2
	14	NT	NT	25(8)	33(12)	NT	
HVP	10	20(20)	50(22)	50(20)	90(20)	100(20)	0.5
	14	NT	NT	NT	75(20)	NT	

[1] NT=Not tested

[2] Percent infected (Number tested) after the indicated number of days of extrinsic incubation at 27°C.

It would appear that our congenic lines are not as genetically pure as we had hoped. We are suggesting that the congenic lines might provide an excellent starting point for the selection of high and low viral producing lines within a short period of time. The reason for the anticipatead rapidity of selection is because these mosquitoes are genetically inbred with a low percentage (15%) revealing some degree of modulation. In addition, since the congenic lines are highly susceptible to peroral infection with WEE virus, this type of selection scheme should hypothetically isolate peroral susceptibility from modulation. This obviously had not occurred in our previous selections (Table 6). We anticipate genetically pure lines within the year.

VI. References Cited

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